BIKEN JOURNAL Vol. 20 91-93, 1977

SHORT COMMUNICATION

POSSIBLE PRESENCE OF A GENUS-SPECIFIC ANTIGEN IN VIBRIOS

YOICHI OKADA and SABURO MIYAMOTO

Department of Microbiology, Osaka City Institute of Public Health and Environmental Sciences, Tojo-cho, Tennoji-ku, Osaka

MASAHIKO YONEDA[1]

Department of Tuberculosis Research I, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita, Osaka

(Received December 10, 1976)

Vibrio parahaemolyticus, first isolated by Fujino et al. (1953), is now known as the most widespread pathogenic agent causing food poisoning in Japan (Takikawa, 1958; Sakazaki et al., 1963). During the course of our study on the serological relationships between this organism and other vibrios, we have recently found that a protein antigen, isolated from the cells of a strain of *V. parahaemolyticus*, appears to be present in the strains belonging to the genus *Vibrio* but not in those of other genera tested.

Antigenic analysis of test strains was made by means of Ouchterlony's immunodiffusion technique (Ouchterlony, 1948) employing the protein antigen, designated as C2, and its specific antiserum as indicators.

C2 antigen was prepared by the following method reported previously (Okada et al., 1966). Thus, from the culture of *V. parahaemolyticus* strain A-55, grown at 37C for 16 hr on a nutrient agar containing 3% sodium chloride, cells were collected, washed twice in a 3% solution of the salt by centrifugation and the washed cell suspension in the saline was then treated in the cold by sonic oscillation at 10 kc for 4 min. The whole disintegrates were then centrifuged at $105{,}000 \times g$ for 120 min and the supernatant thus obtained was fractionated with ammonium sulfate at neutral pH at 50–80% saturation.

Isolation and purification of C2 from the ammonium sulfate fraction were accomplished by a combination of zone-electrophoresis (Yoneda and Fukui, 1961) using starch as the supporting medium, DEAE-cellulose column chromatography (samples were applied to the column in 0.01 M tris(hydroxymethyl) amino methane-HCl buffer, pH 8.1, and the fraction containing C2 was eluted with the buffer containing 0.05 M NaCl) and gel filtration through Sephadex G-75 column (2.5×90 cm; 0.01 M Tris HCl buffer, pH 8.1), yielding the C2 as the highly purified protein preparation, which formed a single precipitation line in an Ouchterlony's plate against the rabbit antiserum for the crude cell-extracts of *V. parahaemolyticus* strain A-55.

The indicator antiserum was obtained by immunizing rabbits with the highly purified preparation of C2 antigen in a Freund's in-

1 Deceased on 29 August 1976.

complete adjuvant. The materials tested for the presence of C2 antigen were the cell disintegrates of the strains to be examined, and they were each prepared from unwashed cells of the culture fully grown on each appropriate medium by sonicating (at 10 kc for 10–20 min in the cold) the cells suspended in a 3% sodium chloride solution or in a physiological saline.

A typical example of our tests in Ouchterlony's plates is illustrated in Fig. 1. In this case, test organisms were each one strain of *V. alginolyticus*, *V. costicolus*, *V. cholerae* (Ogawa), *Desulfovibrio desulfuricans* and of *Aeromonas hydrophila*. Against anti C2 serum, the cell-disintegrates of the *Vibrio* strains tested all formed the single precipitation line which completely fused with that developed with indicator C2 antigen, while no precipitation occurred with the disintegrates of other strains, indicating the presence of C2 antigen in the former three strains and its absence in the latter two (Fig. 1).

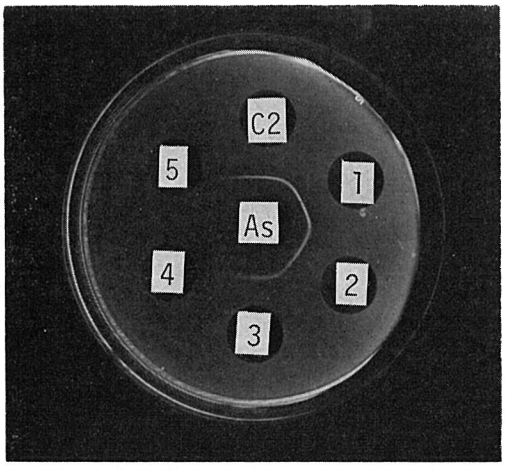

FIGURE 1. Agar gel precipitation pattern demonstrating the presence or absence of C2 antigen in test strains.
C2, C2 antigen (15 μg/ml); As, anti-C2 rabbit serum; cell disintegrates (8.6–16.0 mg protein/ml) of *V. alginolyticus* (1), *V. costicolus* (2), *V. cholerae* (Ogawa) (3), *D. desulfuricans* (4) and *A. hydrophila* (5). Sample volume: 0.2 ml.

TABLE 1. *Distribution of C2 antigen among various bacterial strain*

Test organisms	Number of strains tested	C2 antigen[a]
I. Strains belonging to the genus *Vibrio*		
V. parahaemolyticus	20	+
V. alginolyticus	10	+
V. anguillarum[b]	3	+
V. ichthyodermis[b]	2	+
V. costicolus[b]	1	+
V. piscium[b]	2	+
V. metschnikovii[b]	1	+
V. tyrogenus[b]	1	+
V. cholerae	2	+
V. cholerae (*biotype eltor*)	1	+
V. spp. (Gardner & Venkatraman Group III, V, VI)[b]	4	+
V. spp. (Heiberg)[b]	6	+
V. spp.[c]	8	+
II. Strains belonging to the other genera		
Desulfovibrio desulfuricans[d]	1	−
D. vulgaris[d]	1	−
D. africanus[d]	1	−
Aeromonas hydorophila[c]	3	−
A. formicans[c]	1	−
A. liquefaciens[c]	1	−
Plesimonas shigelloides[c]	1	−
Pseudomonas aeruginosa	2	−
P. fluorescens[e]	1	−
P. putida[e]	1	−
Xanthomonas citri[e]	1	−
X. pruni[e]	1	−
Acetobacter aceti[e]	1	−
A. melanogenum[e]	1	−
Protaminobacter ruber[e]	1	−
Escherichia coli	5	−
Aerobacter aerogenes	1	−
Salmonella typhimurium	1	−
Proteus vulgaris	1	−
Bacillus subtilis	1	−
Corynebacterium diphtheriae	1	−
Staphylococcus aureus	1	−

[a] Presence (+) and absence (−) of C2 antigen.
[b] Strains received from Mr. K. Kotera.
[c] Strains received from Dr. R. Sakazaki.
[d] Strains received from Dr. L. L. Campbell.
[e] Strains received from Institute for Fermentation, Osaka.

Miwatani et al. reported an antigenic substance (A-substance) which was found in all strains of *V. parahaemolyticus*, but not in other species of genus *Vibrio* (Miwatani et al., 1969; Shinoda, Miwatani and Fujino, 1971). On gel diffusion test employing anti-C2 and anti-A-substance rabbit sera, we observed that C2 antigen was antigenically distinct from A-substance.

Table 1 is the summary of the antigenic analyses using C2- anti-C2 serum as a reference system, on the 90 strains of various bacteria including 61 *Vibrio* strains and the 29 strains belonging to the other genera. Without a sole exception, C2 antigen was found in all the *Vibrio* strains tested, but could not be detected in the others tested.

It appears therefore that C2 antigen is distributed among vibrio perhaps as a common antigen specific for the genus *Vibrio*. However, to reach a final conclusion, one must await further studies with more strains. Investigations along this line are now going on in our laboratories.

We thank Drs. Gendo Omori (late) and Junichi Yasuda for advice and encouragement, and to Dr. Riichi Sakazaki, Mr. Kenichi Kotera and Prof. L. Leon Campbell for supplying us the various strains used in this study. We thank Prof. Toshio Miwatani for supplying us A-substance and its anti-serum employed in this study.

REFERENCES

Fujino, T., Y. Okuno, D. Nakada, A. Aoyama, K. Fukai, T. Mukai, and T. Ueho. 1953. On the bacteriological examination of Shirasu food poisoning. Med. J. Osaka Univ. 4: 299–304.

Miwatani, T., S. Shinoda, H. Nishimune, M. Okada, Y. Takeda, and T. Fujino. 1969. A common antigenic substance of *Vibrio parahaemolyticus*. I. Isolation and purification. Biken J. 12: 97–106.

Okada, Y., S. Miyamoto, H. Akehashi, and M. Yoneda. 1966. Studies on common antigens of *Vibrio parahaemolyticus*. Jpn. J. Bacteriol. 21: 490–491 [In Japanese].

Ouchterlony, Ö. 1943. Antigen-antibody reaction in gels. Arkiv. Kemi. Mineral. Geol. 26B: 1–9.

Sakazaki, T., S. Iwanami, and H. Fukumi. 1963. Studies on the enteropathogenic, facultatively halophilic bacteria, *Vibrio parahaemolyticus*, I. Morphological, cultural and biochemical properties and its taxonomical position. Jpn. J. Med. Sci. Biol. 16: 161–188.

Shinoda, S., T. Miwatani, and T. Fujino. 1971. A common antigenic substance of *Vibrio parahaemolyticus*. II. Some physicochemical properties. Biken J. 14: 75–76.

Takikawa, I. 1958. Studies on pathogenic halophilic bacteria. Yokohama Med. Bull, 9: 313–322.

Yoneda, M., and Y. Fukui. 1961. Extracellular proteins of tubercle bacilli. I. Zone-electrophoretic purification of the proteins of a virulent strain (H37Rv) of *Mycobacterium tuberculosis*. Biken's J. 4: 25–40.